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1995 Status Report

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STORED-PRODUCT
INSECTS RESEARCH OF
MILITARY
IMPORTANCE

**Biological Research Unit
U.S. Grain Marketing Research Laboratory
Grain Marketing and Production Research Center
1515 College Avenue
Manhattan, Kansas 66502**

1995 Status Report

"Stored-Product Insects Research of Military Importance"

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U. S. GRAIN MARKETING RESEARCH LABORATORY
GRAIN MARKETING AND PRODUCTION RESEARCH CENTER

LABORATORY MISSION

The mission of the U. S. Grain Marketing and Research Laboratory is to develop new knowledge, information, and technologies needed to solve problems associated with the harvest, storage, marketing, and overall quality and end-use properties of cereal grains. The Laboratory program focuses on post-harvest aspects of grain marketing and includes a major commitment to incorporation of optimum end-use quality characteristics into new grain varieties in cooperation with plant breeders. The Laboratory provides multidisciplinary research on these issues by three research units:

Biological Research Unit
Engineering Research Unit
Grain Quality and Structure Research Unit

MISSION OF THE BIOLOGICAL RESEARCH UNIT

The mission of the Biological Research Unit is to develop new, ecologically-based methods to replace or reduce the use of traditional pesticides for controlling insects in stored grain and stored products. A multidisciplinary team conducts research on 1) novel physiological, genetic, and biological control techniques; 2) improved insecticide deployment and resistance management strategies; 3) the ecology population dynamics, and behavior of pest and beneficial insects; and 4) the development of computer-based integrated pest management systems for farm and commercial grain storage and processing facilities.

We conduct research on biological control agents, insect-resistant packaging, novel physiological control techniques, host plant resistance, insecticide deployment and resistance management strategies, insect biochemistry and genetics, insect population monitoring, population dynamics and behavior, and computer-based integrated pest management systems.

U. S. GRAIN MARKETING RESEARCH LABORATORY

STAFFING/AREA OF RESEARCH EMPHASIS

October, 1995

Acting Center Director

Dr. William H. McGaughey,
Supervisory Res. Entomologist

Microbial Insecticides,
Integrated Pest Management

Biological Research Unit

Dr. Karl J. Kramer, Res. Chemist
Acting Research Leader

Insect Biochemistry and Physiology,
Biopesticides

Dr. Franklin Arthur, Res. Entomologist

Integrated Pest Management
Technologies

Dr. James E. Baker, Res. Entomologist

Parasitoid Biology and Toxicology;
Digestive Physiology

Dr. Richard Beeman, Res. Entomologist

Genetics/Molecular Biology;
Insecticide Resistance

Dr. John H. Brower, Res. Entomologist

Biological Control (Parasites and
Predators)

Dr. Alan Dowdy, Res. Entomologist

Insect Ecology/Behavior in
Commercial Facilities

Dr. Paul Flinn, Res. Biologist

Modeling/Expert Systems

Dr. David Hagstrum, Res. Entomologist

Insect Ecology, Modeling, Sampling,
Acoustic Detection

Dr. Ralph Howard, Res. Chemist

Chemical Ecology; Biological Control

Dr. Donovan Johnson, Res. Microbiologist

Microbiology of Insect Pathogens

Dr. Michael Mullen, Res. Entomologist

Insect Trapping;
Insect Resistant Packaging

Dr. James Throne, Res. Entomologist

Ecology, Modeling,
Seed Resistance to Insects

RESEARCH HIGHLIGHTS

HOST-PARASITOID POPULATION DYNAMICS STUDIED. A population growth model for the predator, *Lyctocoris campestris*, was developed with ARS scientists in Madison, WI. This is an important step toward using this predator for biological control of stored-product insects.

SEMIOCHEMICAL-BEHAVIORAL RELATIONSHIP STUDIED. Cuticular lipids of two parasites and their stored-product insect hosts have been characterized. Experiments in progress are testing the roles of these lipids in species and host recognition processes.

BIOCONTROL OF LESSER GRAIN BORER ACHIEVED. A three year ARS pilot test showed that augmentative releases of hymenopteran parasitoids can suppress lesser grain borer in stored wheat by 88%. Wheat was safely stored for over 195 days, which is longer than most farmers hold their grain.

BIOCONTROL OF INSECT PESTS OF STORED CORN ACHIEVED. Biological control of stored corn insects was shown in a pilot test. A large pilot test involving the release of insect parasites and predators into six 1500 bushel bins of corn was completed at Savannah just prior to closure of the laboratory. Significant decreases of pest insects occurred, providing evidence for the efficacy of this technique for on-farm integrated pest management strategies.

MECHANISM OF INSECT RESISTANCE TO BT IDENTIFIED. Proteinases are involved in insect resistance to BT toxins. The first evidence of a proteinase-mediated response to insecticidal toxins from *Bacillus thuringiensis* (Bt) has been found in the Indianmeal moth, *Plodia interpunctella*. This finding will provide a basis for integrated pest management strategies using Bt insecticidal toxins.

GUT PROTEINASES OF BT SUSCEPTIBLE MOTH CHARACTERIZED. Gut proteinases from a Bt susceptible strain of the Indianmeal moth have been characterized, providing important clues to the role of proteinases in Bt toxicity and adaptation. These findings will be used in the development of effective Bt resistance management strategies.

INSECT POPULATION MODEL IS DEVELOPED. A maize weevil population growth model developed by us was incorporated into an environmental model from scientists at Purdue University. The Purdue scientists are using the coupled model to develop an expert system for training Midwestern corn growers in stored-grain management practices.

COMPUTER PROGRAM FOR BIOASSAY DATA DEVELOPED. A method for analyzing correlated data from serial time bioassays such as pesticide or drug trials, and a computer program for implementing the method were developed. The program makes the method easy to use and provides all output required for proper statistical reporting of the analysis. The work was reported at an international meeting and is being used by other scientists in a number of countries.

SEED RESISTANCE TO INSECTS DISCOVERED. A number of aflatoxin resistant corn lines were shown to vary greatly in their susceptibility to maize weevils but susceptibility was not correlated with chemical or physical properties of lines. These studies will allow lines resistant to both aflatoxin and insects to be selected for commercial development.

SURVIVAL OF INSECTS AT EXTREME TEMPERATURES MODELED. Equations describing survival of lesser grain borer at cold temperatures were developed. Understanding insect responses to extreme temperatures becomes increasingly important as we rely more on ecologically-compatible control strategies such as cooling of the grain using aeration.

INSECT MIGRATION STUDIED. We have research in progress on emigration of insects from grain bins and movement within bins. Both are important in understanding the dynamics of insect colonization of grain.

IMMUNOLOGICAL METHOD TO DETECT HIDDEN INFESTATIONS OF INSECTS IN STORED GRAIN DEVELOPED. An antibody specific for weevil egg plug proteins has been developed for use in a highly sensitive immunological method to detect weevil infestations in stored grain. The method will enable farmers, elevator operators and grain processors to detect weevils right after egg deposition, before population levels reach economic injury levels.

INSECT HORMONES REGULATE INSECT IMMUNE RESPONSES. Insect hormones known as eicosanoids were shown to be important regulators of the ability of a stored grain feeding beetle to mount an immune response against pathogenic bacteria. These findings are the first step in devising strategies to hinder the ability of stored grain insect pests to resist microbial biocontrol strategies.

INSECT HORMONES REGULATE INSECT BODY TEMPERATURES. Insect hormones known as eicosanoids were shown to regulate the ability of an insect to control its internal body temperature. These findings are the first step in devising strategies for hindering the ability of stored grain insect pests to resist control technologies involving high or low grain temperatures.

GENETIC DEFECTS THAT WEAKEN THE EXOSKELETON IN INSECTS IDENTIFIED. The biochemical basis for genetic lesions in insects, which prevent the exoskeleton from being hardened and stabilized, has been delineated. The distribution and metabolism of critical biochemical intermediates used for cuticle formation are obstructed by the mutations. Inhibitors that have similar effects on cuticle metabolism might be developed as insect growth regulators that selectively manipulate cuticle biochemistry for insect pest management purposes.

NOVEL GENES FOR CONTROLLING INSECT PESTS ON PLANTS IDENTIFIED. Although control of insect pests on plants appear possible through the use of transgene technology, one limitation is a shortage of useful genes for this purpose. Several genes have been identified whose encoded protein's target is the insect gut. Some of these genes are being transferred to cereals for use in biotechnological host plant resistance programs to control insect pests of stored grain.

INSECT CHITINASE-MEDIATED RESISTANCE OF TRANSGENIC PLANTS TO INSECTS DEMONSTRATED. An insect chitinase gene has been transferred to tobacco and transgenic plants constitutively expressing the enzyme exhibited enhanced resistance to tobacco budworms. Larval growth rate and survival were significantly reduced. Use of the insect-derived chitinase gene for insect control represents a promising alternative to the use of other transgenes and chemical insecticides.

NEW SULFUR-CONTAINING PRO-INSECT GROWTH REGULATORS DEVELOPED. In searching for selective anti-insect agents, we synthesized and evaluated a series of new derivatives of carbamate-type juvenile hormone mimics. Laboratory and field evaluations against several insect species revealed improved biological properties relative to the parent compound (fenoxycarb). These derivatives might be developed as insect growth regulators that selectively manipulate hormone levels for insect pest management purposes.

CUTICULAR WAXES OF STORED GRAIN PEST TRACK ENVIRONMENTAL STRESSES. Cuticular waxes of larvae and adults of the saw-toothed grain beetle were shown to change in the presence of desiccants or low temperatures. These findings are of importance in developing on-farm management strategies involving the use of aeration, desiccants and temperature extremes.

NATURALLY-OCCURRING "SELFISH" GENE KILLS INSECTS. A naturally-occurring gene that regulates pest insect populations was discovered in the U.S. The gene, which can be maternally activated to kill developing eggs, is prevalent in natural populations from the Midwest and Northern Plains, but is absent in Gulf Coast populations. The gene might be useful both for monitoring infestations and for genetically disrupting growth of pest insect populations.

NEW, VIRUS-LIKE GENES FOUND NATURALLY-OCCURRING IN PEST INSECT CHROMOSOMES. A new virus-like gene called a "retrotransposon" was found inserted into the chromosomes of flour beetles. Molecularly engineered versions of this gene might be used as carriers to "infect" wild populations of pests with insect control genes.

FOREIGN GENES TRANSFERRED INTO INSECT CHROMOSOMES USING A BROAD-SPECTRUM RETROVIRAL VECTOR. For the first time, foreign genes have been successfully transferred into insect chromosomes using a retroviral vector. Foreign (bacterial) indicator genes were inserted into a modified retrovirus, which was then used to infect insect cells in culture. The foreign genes functioned normally in the insect host chromosomes. The retroviral vector is now being tested for gene transfer in live insects. This research could lead to a universal gene transfer vector for genetic manipulation of both pestiferous and beneficial insects.

TEST-TUBE ASSAY FOR INSECTICIDE RESISTANCE DEVELOPED. A polymerase chain reaction (PCR)-based assay to detect lindane resistance in single, dead or live insects was developed. The method is based on molecular cloning and detailed DNA sequence analysis of the resistance gene. It can be used to monitor the occurrence or recurrence of insecticide resistance in field populations of pest and beneficial insects.

INSECTICIDE RESISTANCE IN BENEFICIAL INSECTS DOCUMENTED. Two naturally occurring insecticide-resistant strains of beneficial insects, a hymenopterous parasitoid and a predaceous bug, were discovered in grain bins located in the southeast region of the U.S. They are being developed for use in biological control programs for stored product insect pests, and would be compatible with control methods that use insecticidal chemicals.

BT SPORES SYNERGIZE BT TOXINS. Spores of *B.t.* subsp. *kurstaki* were found to synergize crystal protein toxicity for susceptible and certain *B.t.*-resistant strains of *Plodia interpunctella*. These results argue for the use of complete spore-crystal mixtures for biological control of insects, instead of bioengineered products that contain only crystal protein.

ACTIVITY OF INSECT BIOCONTROL AGENT ENHANCED BY GENETIC TRANSFORMATION WITH AN INSECT MOLTING ENZYME GENE. ARS scientists, in cooperation with scientists at Kansas State University, have been working to enhance the activity of biological control agents used for insect pest management. The gene for an insect molting enzyme called chitinase was cloned and transferred to an entomopathogenic virus. The recombinant virus expressed the active enzyme and killed insects more rapidly than the wild-type virus. The gene for this enzyme has the potential to be manipulated by the agricultural biotechnology industry for the improvement of microbial pesticides.

HOST PLANT RESISTANCE TO INSECT PESTS ENHANCED BY GENETIC TRANSFORMATION WITH AN INSECT MOLTING ENZYME GENE. ARS scientists, in cooperation with scientists at Kansas State University, have been working to enhance the resistance of plants to insects using chemical defense genes. The gene for an insect molting enzyme chitinase was cloned and transferred to tobacco. Transgenic tobacco plants expressing the enzyme were more resistant to insect pests than plants not expressing the enzyme. The gene for this enzyme has the potential to be manipulated by the agricultural biotechnology industry for the improvement of host plant resistance to insects.

SOURCES OF INSECT INFESTATION IN FARM AND COMMERCIAL STORAGE IDENTIFIED. Research was continued on the nature and sources of insect infestation around grain storage facilities to try to understand where infestations originate. This is important to minimize chemical usage and better target control strategies.

THE ORIGIN OF INDIANMEAL MOTH POPULATIONS CAN BE IDENTIFIED USING DNA FINGERPRINTING TECHNIQUES. This may eventually be adapted for determining at what point in the marketing channel a commodity became infested.

NEW INSECT TRAPS DEVELOPED. In a continuing research project, the development of new pheromone baited traps for stored product insects is being studied. Currently under development is an inexpensive disposable pheromone trap that can be packed with cargo at the time of shipment and checked at the final destination. Use of this trap will provide a means of determining infestation in exported and imported cargo so that appropriate remediation can be instituted if necessary.

EXPERT SYSTEM FOR GRAIN MANAGERS DEVELOPED. Stored Grain Advisor (SGA), an expert system that helps farmers manage insect pests of stored wheat, was developed and field tested. SGA reliably predicted the level of infestation. SGA is available from the Cooperative Extension Service at Kansas State University, Oklahoma State University and Montana State University.

FALL TEMPERATURES PERMIT EXPANDED USE OF AERATION FOR CORN AND WHEAT STORED IN THE SOUTHEAST. Results of several studies show that aeration will limit insect pest populations in corn and wheat stored in the Southeastern United States. There is considerable potential for the expanded use of aeration in management programs. Successful implementation of new aeration strategies could lead to a reduction in the amount of pesticides used in stored commodities.

PARASITOIDS CONTROL INSECTS IN STORED GRAIN. Parasitoids were shown to be effective in reducing rusty grain beetle and lesser grain borer infestations. Models were developed which will allow recommendations for using parasitoids to be provided by the expert system.

AUTOMATED INSECT MONITOR DEVELOPED. An automated system for continuously monitoring insect populations in stored grain with acoustical sensors was developed and field tested. The automated system detected insects in all of the bins in which insects were found in grain samples and provided a good estimate of infestation level.

INSECT POPULATION GROWTH MODEL DEVELOPED. A bin temperature and an insect population growth model were coupled to more accurately predict insect population growth in the fall and spring when temperature gradients exist between locations in the grain mass. The accuracy of predictions was further improved by collecting and incorporating new cold temperature survival data for lesser grain borer.

RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-014-00D

Title: Ecology, Modeling, and Integrated Management of
Stored-Product Insects

Main Objectives:

Pest management decisions can be improved by developing better insect monitoring programs and models for predicting insect population growth. Research will provide the technology that grain managers need to detect insect infestations more accurately and earlier, predict insect population growth rates and effects of different control measures, and forecast when insect control will be needed. These management tools are needed for a transition to less chemical dependent integrated pest management programs. Project focuses on acquiring essential biological and ecological data, using these data to develop predictive models of insect population growth, and developing an expert system for stored grain management that uses the models. Studies will expand the range of species and conditions over which existing models predict insect population growth and the range of problems for which the expert system can make pest management recommendations. Expansions will include additional pest species, natural enemies, grain varieties, aeration, cold temperature survival, and insect movement. Improved sampling programs will be developed to collect ecological data and acquire information needed to make pest management decisions. Automatic insect monitoring using acoustical sensors will be investigated.

Investigators: David W. Hagstrum, Paul W. Flinn,
James E. Throne, Charles S. Burks

1. Specific Objective: Quantify effects of temperature and moisture on fecundity of maize weevils for inclusion in maize weevil model.

Progress FY95: Continued cooperative study to develop ELIFA method for quantifying weevil egg plugs.

Plan of Work FY96: Continue development of ELIFA method.

2. **Specific Objective:** Modify and validate computer model for simulating maize weevil population dynamics.

Progress FY95: Data describing the effects of temperature and moisture on development of immature maize weevils was incorporated into the model. The maize weevil model was incorporated into a corn ecosystem model developed at Purdue University. The resulting model was used to evaluate various strategies for corn storage, particularly comparing the effects of ambient aeration and grain chilling.

Plan of Work FY96: Incorporate new data as they become available, including fecundity data, effects of temperature and moisture on development of individual stages of maize weevils, and effects of corn variety on weevil development.

3. **Specific Objective:** Determine relative resistance to maize weevils of corn lines bred for their resistance to an aflatoxin-producing fungus. Correlate maize weevil development with physical and chemical properties of the corn lines.

Progress FY95: A number of corn lines that had previously been shown to be relatively resistant to infection by aflatoxin-producing fungi (by scientists with ARS in MS) were shown to vary greatly in their susceptibility to maize weevils. However, susceptibility to maize weevils was not correlated with chemical or physical properties of the corn lines. The corn lines that were found to be relatively resistant to both aflatoxin-producing fungi and maize weevils are most promising for commercial development.

Plan of Work FY96: This study has been completed.

4. **Specific Objective:** Develop a method for analyzing serial time-mortality data.

Progress FY95: Serial time-mortality data result from bioassays when mortality due to one dose of an insecticide is determined at several times. These data cannot be analyzed using standard probit analysis programs because the observations are correlated. We developed a method for analyzing such data, and developed a computer program for implementing the method. Companion programs that can be used with output from any probit analysis program were written to: 1) backtransform probit-transformed data to aid in assessing goodness-of-fit, 2) compare slopes and intercepts from two probit lines, and 3) determine relative potency of two compounds.

Plan of Work FY96: This study has been completed.

5. Specific Objective: Field testing of reliability of expert system, Stored Grain Advisor.

Progress FY95: Stored Grain Advisor (SGA) for farm-stored grain was validated using three years of field data from over 50 grain bins. Bins of newly-stored wheat were sampled monthly from June through January for insects, grain temperature, moisture, test weight and insecticide residue. SGA was correct 80 percent of the time in predicting when bins would become infested with low, moderate or high insect densities.

Plan of Work FY96: Additional improvements will be made in Stored Grain Advisor for the farm and it will be modified for use at grain elevators.

6. Specific Objective: Develop a compartmented model to improve prediction of insect population growth in fall and spring when temperature gradients exist between locations in grain mass.

Progress FY95: A spatial model describing insect population dynamics in a grain bin was developed by coupling a rusty grain beetle population growth model with a two dimensional bin temperature model that predicts seasonal changes in grain temperature for any location within a grain bin. Field data for a 10,000 bushel bin of wheat located in Cloud County, Kansas was used to validate the model. During Fall in unaerated grain, both the field data and our model showed that the outer layers of the grain mass cool more rapidly than the center layers. Because grain insects increase more rapidly at warmer than at cooler temperatures, grain insects reached higher densities in the center than in the outer layers of the grain mass. The model predicted both the number of insects and the grain temperature accurately during the storage season.

Plan of Work FY96: The behavior of insects in temperature gradients will be studied to predict movement of insects between compartments.

7. Specific Objective: Develop and validate a population growth model for a parasitoid of the rusty grain beetle.

Progress FY95: Completed a mathematical computer model of the hymenopteran parasite *Cephalonomia waterstoni*, and coupled it with a model of the rusty grain beetle that we had previously developed. In validation studies, the model accurately predicted both the time and magnitude of peak parasitoid density.

Simulations with the model showed that changing the timing of parasite release had a greater effect than releasing more parasites. Adding four times as many parasites only decreased the maximum host population by 40%. Releasing parasites at day 25 instead of day 45 reduced the maximum host population by 75%. Timing of release depends on parasites finding the first-produced fourth instar larvae; which depends on grain temperature and moisture.

Plan of Work FY96: Model will be developed and validated for parasitoid of lesser grain borer.

8. Specific Objective: Field testing of efficacy of lesser grain borer and rusty grain beetle parasitoids.

Progress FY95: Field studies were conducted to assess the effectiveness of the parasitoid wasps, *Cephalonomia waterstoni* for controlling rusty grain beetle and *Choetospila elegans* for controlling lesser grain borer. The amount of suppression of rusty grain beetle by *C. waterstoni* could not be measured in this study because large numbers of *C. waterstoni* entered the control bins. *Choetospila elegans* was very effective and suppressed lesser grain borer populations 91%.

Plan of Work FY96: Information on using parasitoids will be included in the expert system, Stored Grain Advisor.

9. Specific Objective: Automation of insect monitoring in farm stored wheat using acoustical sensors on cables.

Progress FY95: An automated method using cables with acoustical sensors was compared with the conventional grain sampling method for monitoring insect populations in wheat stored in 1 or 2 bins on each of 6 farms in Kansas. Seven flexible cables each with 20 sensors 15 cm apart were installed vertically in the grain mass along a transect across the diameter of the bin. A computer collected and stored the data. The automated system detected insects in all of the bins in which insects were found in grain samples and provided a good estimate of infestation level.

Plan of Work FY96: We are working with The Rolfes company and scientists at ARS laboratory in Gainesville, FL to develop a better cable.

10. Specific Objective: Model cold temperature survival of insects in stored wheat.

Progress FY95: Cold temperature survivorship of lesser grain borer populations with a natural age structure in stored wheat was estimated for a natural fall cooling rate of 2°C/wk and winter temperatures of 17, 13, 9 and 5°C. The colder the temperature the more rapidly survivorship decreased. The mostly immature insect population inside kernels was killed less rapidly than the external adult population at 5 and 9°C and more rapidly at 13 and 17°C. Daily survival rates decreased and then increased again indicating that those insects surviving short exposures to cold temperatures had a greater chance of surviving long exposures to cold temperatures. These equations will help extend our population model to predict the survival of lesser grain borer populations in stored wheat through the winter.

Plan of Work FY96: Models will be developed for additional species and the physiological basis of cold hardiness will be studied to find better ways to model cold temperature survival.

11. Specific Objective: Monitoring infestation of newly harvested wheat by insects using headspace sticky traps.

Progress FY95: Insect populations in 14 bins of newly-harvested wheat on eight farms in Kansas were monitored with sticky traps in the bin headspace and with grain samples. Sticky trap catches during the first three weeks of storage were used to provide an estimate of the species and densities of insects that were present in the headspace. Grain samples were taken every two weeks during the first three months of storage to provide an estimate of population growth under the grain temperature and moisture conditions in the bins. The sticky traps correctly predicted whether lesser grain borers and rusty grain beetles would be found in the grain samples. The total numbers of rusty grain beetle adults in the grain samples could be predicted better from the product of mean grain temperature times maximum grain moisture than from sticky trap catch.

Plan of Work FY96: Several types of traps will be used to develop a better understanding of the phenology of infestation of newly harvested wheat.

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RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-015-00D

Title: Biological Control of Stored Product Insects With Parasites, Predators and Entomopathogens

Main Objectives:

The goal of this project is to develop economically viable pest management systems that capitalize on desirable biochemical, behavioral and biological traits of parasites, predators and entomopathogens of stored grain insect pests. Specific objectives include characterizing life histories of promising parasites and predators exposed singly and in combination to single- and multiple-species combinations of stored product pests; identifying the semiochemical and behavioral mechanisms used by these biological control agents to locate, recognize and kill their hosts; characterizing behavioral and semiochemical responses by pests that ameliorate the effectiveness of biological control agents; assessing specific stress factors such as temperature, relative humidity, desiccants and inhibitors of insect-specific hormones that might weaken pests and make them more susceptible to biocontrol agents; determining parasite-host release ratios, timing of releases, and effect of multiple-species releases on control efficacy; evaluating possible detrimental effects of releasing biological control agents into stored grain commodities; elucidating the biochemical mechanisms by which the toxins of *Bacillus thuringiensis* (*B.t.*) kill stored product pests, with particular emphasis on the physiology of membrane receptor-protein interactions; evaluating the extent of resistance and cross-resistance to native, cloned, and truncated *B.t.* toxins in stored product insect populations; and characterizing insect resistance mechanisms involving the interaction of *B.t.* toxin proteins with midgut proteinases in *Plodia interpunctella*.

Investigators: Ralph W. Howard, John H. Brower, Donovan E. Johnson, William H. McGaughey, Brenda Oppert

1. **Specific objective:** Identify cuticular hydrocarbons from the parasite *Cephalonomia stephanoderis* and its beetle host.

Progress FY95: Hydrocarbons were identified and compared to the stored product parasite *C. waterstoni* and its beetle host that we had earlier identified. Substantial differences were found between the two parasites and between the two hosts, but

both showed strong sex-related differences that suggest that their hydrocarbons are being used for species and gender recognition.

Plan of work FY96: Complete analysis and report the work in the Bulletin of Entomological Research.

2. Specific Objective: Determine the mechanisms by which *Cephalonomia waterstoni* and *C. tarsalis* recognize their specific hosts, the rusty grain beetle and the saw-toothed grain beetle, respectively.

Progress FY95: Cultures of both parasites and their hosts were established. Protocols for conducting detailed video behavioral analyses were established.

Plan of Work FY96: Conduct detailed behavioral analyses and generate an ethogram for host recognition by these parasites. Using these ethograms, develop bioassays to separate chemical from other possible cues.

3. Specific Objective: Evaluate the possible roles of pheromones in sexual behavior by the parasitoid *Anisopteromalus calandrae*.

Progress FY95: The parasite and its host, the rice weevil, were established in cultures. Video protocols were established for generating ethograms.

Plan of work FY96: Conduct detailed behavioral analyses of the courtship and copulatory behavior of the parasite. Using the developed ethograms, develop bioassays to separate chemical from other cues. Begin isolation of possible pheromones.

4. Specific Objective:

To determine the effectiveness of the parasite, *Pteromalus cerealellae*, in reducing populations of the Angoumois grain moth, *Sitotroga cerealella*, on both wheat and corn.

Progress FY95: The degree of control obtained depended on both host and parasite densities. The greater the density of the parasites with respect to the hosts, the greater the control of the moth.

Plan of Work FY96: The biology and population dynamics of this parasite will be studied in wheat and the intrinsic rate of increase determined under various environmental conditions.

5. **Specific Objective:** To test the acceptance of the Angoumois grain moth as a host for the weevil parasitoid, *Anisopteromalus calandrae*, in the presence or absence of their preferred host, *Sitophilus* weevils.

Progress FY95: Established cultures of *A. calandrae* on the rice weevil in wheat.

Plan of Work FY96: Screen different age cultures of the Angoumois grain moth for attack by *A. calandrae*, with and without the presence of rice weevils.

6. **Specific Objective:** To determine the degree of interspecific competition between two parasitoids, *Anisopteromalus calandrae* and *Pteromalus cereallae* in the presence or absence of rice weevils or the Angoumois grain moth.

Progress FY95: Established colonies of the two hosts and the two parasitoids on hard red winter wheat.

Plan of Work FY96: Conduct a large multifactorial test of the interactions among the two parasitoids and their hosts.

7. **Specific Objective:** Determine the density of multiple beneficials needed to reduce multiple host populations to acceptable levels in six metal grain bins filled with corn.

Progress FY95: The second year of a large biological control pilot project was successfully completed. Pest populations were monitored using several techniques and the data are being summarized for analysis. Preliminary results indicate that control of the different pest populations ranged from poor to excellent depending on species.

Plan of Work FY96: None.

8. **Specific Objective:** Characterize insect gut proteinase interactions with the insecticidal proteins isolated from *B.t.*

Progress FY95: *B.t.* susceptible and resistant strains of the Indianmeal moth, *Plodia interpunctella*, were screened for overall gut proteinase activity and for their ability to activate *B.t.* toxins. Three strains were selected for use in further investigations: one was a *B.t.* susceptible strain, one was a strain resistant to *B.t.* subspecies *kurstaki* which is suspected to be resistant due to a change in receptor binding, and one was a strain resistant to *B.t.* subspecies *entomocidus* which

showed dramatically lower gut proteinase activity. These strains were characterized by their overall gut proteinase activities using general and specific enzyme substrates and inhibitors. Enzyme activity in the *entomocidus*-resistant strain was lower than the susceptible or *kurstaki*-resistant strain. Enzymes from the *entomocidus* strain were unable to fully activate *B.t.* toxins, indicating a possible link between the lower gut activity and *B.t.* resistance in this strain. The initial findings from this work have been published, and a more extensive evaluation of overall gut activity in these strains is being readied for publication.

Plan of Work FY96: Insect proteinases will be isolated and characterized by their interactions with *B.t.* proteinases. Genes for these proteinases will be cloned, allowing for analyses of gene structure and expression in various insect strains. Once proteinase genes are available, translation in an expression system will allow us to obtain larger quantities of proteinases. This will allow us to generate antibodies to these proteinases, which will then allow for specific gut localization studies.

9. Specific Objective: Determine effects of spores on entomocidal activity of CryIAb and CryIC crystal proteins from *B.t.* toward the Indianmeal moth.

Progress FY95: Spores from *B.t.* were synergistic for crystal protein activity towards susceptible Indianmeal moth larvae. Spores also synergized Cry protein activity for certain types of Indianmeal moth larvae resistant to *B.t.* varieties *aizawai* and *entomocidus*, but not larvae resistant to *B.t.* subsp. *kurstaki*.

Plan of work FY96: Investigate the role of the spore as a synergist. Does increased activity arise from the contribution of spore coat protein toxicity, or does increased activity come from spore germination in the hemocoel and subsequent septicemia within the larvae?

10. Specific Objective: Determine membrane binding characteristics of susceptible and *B.t.*-resistant Indianmeal moth for crystal proteins from *B.t.*

Progress FY95: Evidence of binding of *B.t.* Cry protein toxins to specific receptor proteins from *Plodia interpunctella* midgut brush border membrane vesicles has been shown. At present, binding is very weak and only to CryIAc protein. Work is underway to alter techniques to improve sensitivity of the detection methods.

Plan of Work FY96: Continue technique improvement studies until sufficient sensitivity is achieved. Comparisons of binding proteins from susceptible and *B.t.*-resistant Indianmeal moth larvae will then be examined.

11. Specific Objective: Determine if there is a genetic linkage between *B.t.* susceptibility and proteinase activity in *Plodia interpunctella*.

Progress FY95: Studies were initiated with susceptible and resistant insects to evaluate the minimum dose of *B.t.* necessary to elicit a difference in proteinase activity.

Plan of Work FY96: Resistant and susceptible insects will be mated and backcrossed to the resistant line in order to obtain a homogeneous population of resistant insects. Bioassays will be conducted on each line in order to compare toxicity.

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RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-016-00D

TITLE: Development of Physiological and Genetic Controls for
Stored Product Insects

Main Objectives:

The goals of this project are to characterize physiological and genetic processes that can be manipulated for insect control purposes, identify inhibitory proteins and genetically modify cereal grains and entomopathogens with their genes, develop techniques for genetic manipulation of insects, devise genetic methods for managing pesticide resistance in insects and for enhancing the efficacy of beneficial insects. Specific objectives include identifying target sites in insect skeletal, gut and endocrine systems vulnerable to biopesticides; characterizing molting and digestive enzymes, identifying inhibitors whose genes are amenable to plant and microbial genetic engineering; evaluating recombinant seeds for resistance to insects and evaluate recombinant microbial pathogens for efficacy as biological control agents; identifying inhibitors of insect hormones, evaluating modes of action and efficacy as regulators of growth and physiological processes; characterizing cuticular components of pests and parasites, assessing functional responses to environmental stress; characterizing genetic mechanisms regulating reproduction, development and pesticide resistance of pest and beneficial insects; and developing techniques for genetically altering, manipulating and monitoring populations of pest and beneficial insects.

Investigators: Karl J. Kramer, James E. Baker,
Richard W. Beeman, Ralph W. Howard

1. Specific Objectives: Identify potential chemical defense proteins and screen them for their ability to disrupt stored grain insect development and physiology.

Progress FY95: A baculovirus-insect cell line gene expression system was developed for production of large quantities of potential defense-related proteins, which will be used to determine their spectra of insecticidal activity when administered orally. An insect chitinase gene was found to increase the killing rate of a recombinant nonoccluded baculovirus. Tobacco plants were transformed with vectors containing the insect chitinase gene and resistance of the recombinant

plants to budworms was demonstrated. A patent application for recombinant insect chitinase and its use as a biocide was submitted. Several proteinaceous inhibitors of insect amylases and proteinases were screened for growth inhibiting activity. Combinations of inhibitors from potato, rice and kidney bean were most inhibitory.

Plan of Work FY96: Continue studies on the development of several proteins that disrupt insect gut and cuticle physiology for use as chemical defense proteins.

2. Specific objectives: Characterize reactants, intermediates, products and enzymes responsible for catecholamine metabolism in insect cuticle sclerotization, which might be disrupted by novel insect-selective control agents.

Progress FY95: The biochemical basis for a genetic defect that leads to a weakened and white-colored cuticle in insects was determined. The mechanism for reactions between cuticle cross-linking agents and protein model nucleophiles and the structures of major products from the reactions of quinones with N-acyl derivatives of cysteine and histidine were elucidated.

Plan of Work FY96: Continue to identify insect-specific reactions that occur in the insect cuticle that could serve as targets for new insect growth regulators.

3. Specific Objective: Clone insect chitinolytic enzyme genes and study their molecular biology to determine how insects regulate chitin degradation during molting. Use chitinolytic enzymes as chemical defense proteins to help control pest insects by manipulating their genes in transgenic plants and baculovirus vectors.

Progress FY95: The characterization of an insect chitinase cDNA was finished and the characterization of a genomic clone for chitinase was nearly completed. The 15 kb genomic clone is organized into 10 exons and 10 introns. A full length cDNA clone for insect β -N-acetylglucosaminidase was isolated.

Plan of Work FY96: Finish characterization of β -N-acetylglucosaminidase gene. Isolate samples of recombinant chitinase and β -N-acetylglucosaminidase for bioassays against stored product pests.

4. Specific Objective: Evaluate the efficacy of a malathion-resistant strain of *Anisopteromalus calandrae* as a parasitoid of rice weevils in wheat treated with different concentrations of malathion.

Progress FY95: Based on dose response and serial time-response bioassays, malathion concentration had no significant effect on longevity, fecundity, or

effectiveness of the Bamberg strain of *A. calandrae* parasitizing *S. oryzae* in wheat. Suppression of the immature weevil population exceeded 90% on malathion-treated wheat. Bamberg *A. calandrae* were more successful parasitizing the Savannah laboratory strain of *S. oryzae* compared with the Bamberg strain of *S. oryzae*, possibly because of the larger size of the Savannah weevils. The Bamberg strain of *S. oryzae* (12 times resistant at the LD₉₉ based on vial bioassays) was more tolerant of malathion applied to wheat than was the susceptible Savannah strain of *S. oryzae*. However, malathion concentration had no significant effect on emergence of adults of either weevil strain when wheat containing immatures was treated. Although oviposition was significantly reduced, both weevil strains oviposited on wheat treated with malathion. These results indicate that the ecology of host development (for example, protected weevil larvae feeding within grain kernels) may be primarily responsible for development of the resistance in Bamberg *A. calandrae* by providing a food source when the parasitoid is under selection pressure.

Plan of Work FY96: Continue efforts to study the interaction of parasitoids and pesticides in the stored grain ecosystem.

5. Specific Objective: Determine stability of malathion resistance in two hymenopterous parasitoids.

Progress FY95: The malathion resistance documented in *A. calandrae* that was collected in September 1992 near Bamberg, SC was stable after 23 generations of laboratory rearing with no selection pressure. F₂₃ progeny of this parasitoid strain were 2,800-fold resistant to malathion relative to a susceptible laboratory (Savannah) strain and 200-fold more tolerant of malathion than the field strain of the insect host, the rice weevil, *Sitophilus oryzae* with which they were collected. Malathion resistance in F₂₁ male and female progeny of a field strain of *Bracon* [*Habrobracon*] *hebetor* collected in South Carolina was not significantly different from that of F₆ progeny. However, LT₅₀'s determined in a serial-time bioassay were 0.2-fold lower and may indicate a slight decline in resistance following 15 generations of laboratory rearing.

Plan of Work FY96: Collect field strains of parasitoids in Kansas and Oklahoma and evaluate these strains for their sensitivity to protectants used in the Midwest grain belt.

6. Specific Objective: Evaluate resistance levels in the Bamberg field strain of *A. calandrae* after laboratory selection with 3 grain protectants.

Progress FY95: Seven cycles of laboratory selection with malathion, chlorpyrifos-methyl, and pirimiphos-methyl did not increase resistance levels in the Bamberg strain of *A. calandrae* to these grain protectants. Resistance levels against chlorpyrifos-methyl (5-fold) and pirimiphos-methyl (7-fold) were nearly identical to that measured immediately after the strain was collected.

Plan of Work FY96: Field strains of parasitoids from the Midwest grain belt, if available, will be exposed to insecticide treatments in the laboratory in an attempt to increase the frequency of resistance genes in each population.

7. Specific Objective: Determine mechanisms of resistance of *A. calandrae* to grain protectants.

Progress FY95: In cooperation with Dr. Richard Beeman, experiments have been initiated to determine the mechanisms of inheritance of the malathion resistance in the Bamberg strain of *A. calandrae*. In cooperation with Drs. Alan Dowdy and Yu-Cheng Zhu, studies on molecular differences between the resistant and susceptible strains of *A. calandrae* have been initiated.

Plan of Work FY96: Continue efforts to understand the genetic, biochemical, and molecular mechanisms of malathion resistance in the hymenopterous parasitoid *A. calandrae*.

8. Specific Objective: Analyze dill cultivars (as part of a cooperative research Capacity Building Grant awarded to Southern University and A&M College, Baton Rouge, LA).

Progress FY95: Essential oil components of approximately 110 dill samples (1994 crop) were analyzed by GC and GC/MS. An extensive report detailing the percent composition of the 4 major components [α -phellandrene, limonene + β -phellandrene, anethofuran (3,6-dimethyl-2,3,3A,4,5,7A-hexahydrobenzofuran), and carvone] in each sample was prepared and forwarded to the research PI.

Plan of Work FY96: Prepare grant summary.

9. Specific Objective: Develop methodology for genetic manipulation of pest insects.

Progress FY95: A retroviral-based gene vector was successfully integrated into identified chromosomal sites in cells of the mosquito, *Anopheles gambiae*.

Plan of Work FY96: Work is now underway to use the retroviral vector for gene transfer in flour beetles and other pest insects. Expected benefits include the ability to infect pest species with insect control genes (viruses, lethal genes, transposons), transfer of pesticide resistance genes to parasitoids, and transfer of deleterious traits (such as disease susceptibility, pesticide susceptibility and cold intolerance) to pest species.

10. Specific Objective: Characterize insect resistance to grain protectants.

Progress FY95: A DNA-based test for diagnosis of lindane resistance in single insects was developed using the polymerase chain reaction (PCR), and used for resistance diagnosis in flour beetles. Results of the PCR assay correlated well with bioassay data.

Plan of Work FY96: Genetic and molecular characterization of pyrethroid, *Bt* and malathion resistance genes is in progress.

11. Specific Objective: Use genetic markers to identify insect subpopulation infestation sources.

Progress FY95: Flour beetles were collected from elevators & mills throughout southern & midwestern states. Genetic markers were used successfully to identify and differentiate "gulf coast" and "midwestern" subpopulations of flour beetles, previously not known to exist.

Plan of Work FY96: The exact boundaries of the subpopulations, as well as potential mixing zones, will be identified by more extensive sampling.

12. Specific Objective: Harness natural insect control genes.

Progress FY95: The global distribution of a family of naturally-occurring killer genes was determined in flour beetle populations. The genes were common in the U. S., South America, Africa and Southeast Asia, but were rare or absent in India and Australia.

Plan of Work FY96: Work toward cloning and characterizing the killer genes has been initiated.

13. Specific Objective: Evaluate the capability of the tenebrionid beetle model stored product pest *Zophobas morio* to produce nodules when challenged with a bacterial infection, and determine if nodule formation is regulated by eicosanoids.

Progress FY95: Last instar *Z. morio* larvae were shown to produce nodules when infected with the pathogenic bacterium *Serratia marcescens*. The beetle was also shown to contain eicosanoids and that when those eicosanoids were inhibited, then the insect did not produce nodules in response to bacterial infection.

Plan of work FY96: Examine the other life stages of *Z. morio* for nodule production and begin studies to assess which eicosanoids are involved in the nodulation process.

14. Specific Objective: Evaluate the changes in cuticular hydrocarbon composition of larvae and adults of the saw-toothed grain beetle when exposed to low temperatures of desiccants.

Progress FY95: Both larvae and adults were shown to markedly alter their cuticular hydrocarbon profiles in response to environmental stresses.

Plan of Work FY96: Extend the range of environmental variables these beetles are exposed to and evaluate their hydrocarbon changes to these stresses.

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RESEARCH PROGRESS AND PLANS

CRIS No. 5430-43000-017-00D

Title: Monitoring and control strategies for stored-product insects

Main objectives:

The overall objective of this CRIS is to develop monitoring and control strategies for stored-product insect pests in and around storage and processing facilities. These studies will lead to the development and improvement of genetic, semiochemical and physical techniques to monitor insect behavior, estimate population densities, and determine control thresholds and timing of management actions. Selected control tactics will be evaluated for integration into ecologically-compatible pest management programs. Insect resistant packaging and barriers will be developed to prevent infestation of stored products. The acquired knowledge will lay the ground work necessary to evaluate the efficacy of current practices and to develop ecologically-sound insect pest management programs.

Investigators: Michael A. Mullen, Franklin H. Arthur,
Alan K. Dowdy, Yu-Cheng Zhu

1. Specific Objective: Development of a disposable pheromone baited trap for stored-product Coleoptera.

Progress FY95: Laboratory testing of a disposable pheromone trap made from a plastic vial was completed. It was determined that the shape of the access holes to the trap was not critical in trap catch. The trap was found to be very effective in capturing adults of the red flour beetle.

Plan of Work FY96: The trap will be modified as suggested by laboratory testing and final testing will be completed under field conditions.

2. Specific Objective: Evaluation of pheromone formulations for the sawtoothed grain beetle.

Progress FY95: Five pheromone lures were evaluated and it was determined that no significant differences were found to exist between the lures.

Plan of Work FY96: Tests will be refined and lures will be compared on a one to one basis to attempt to determine if any differences do exist. Once the best lure is determined tests to establish the longevity will be conducted.

3. Specific Objective: Redesign the FLIT-TRAK M² to improve the rate of catch and ease of counting trapped insects.

Progress FY95: A redesign of the trap was discussed with the manufacturer and testing of several prototypes was started.

Plan of Work FY96: Testing of prototypes will be continued. A study to determine the ease of counting trapped insects will be completed.

4. Specific Objective: Use of pheromone traps as a dispersal mechanism for granulosis virus.

Progress FY95: A testing procedure was determined for using baited pheromone traps to disperse granulosis virus.

Plan of Work FY96: Use of pheromone traps to disperse the granulosis virus will continue and technique will be refined.

5. Specific Objective: Improvement of seals and closures on commercial packaging to reduce infestation by stored-product insects.

Progress FY95: Cooperation with food processing companies was re-established. Five test chambers 10 x 20 x 8 ft were designed and constructed. Tests for several companies were conducted. A new more expensive package for raisins was tested and determined not to be better than the existing package. This resulted in the packager not having to retool for the new package and resulted in significant savings. In cooperation with several processors dry pet food packages were tested to determine how seams and closures could be improved to reduce insect infestation.

Plan of Work FY96: Testing of packages will be continued and recommendations will be made to the manufacturers to improve insect resistance.

6. Specific Objective: Test chemical odor neutralizers for use in insect resistant packaging.

Progress FY95: A bioassay was developed to test the odor neutralizer. Tests were initiated to determine the effectiveness of the material.

Plan of Work FY96: Testing of the material will continue. In cooperation with the manufacturer this material will be incorporated into different packaging materials to determine its usefulness in commercial packaging.

7. Specific Objective: Evaluation of residual insecticides on different flooring surfaces.

Progress FY95: Several insecticides have been tested on concrete, floor tile, metal, and wood. Most of these insecticides have greater residual persistence on non-porous surfaces. The cyfluthrin WP formulation is more effective than the EC formulation when applied to concrete. Different paints used by the Armed Forces also affect residual efficacy of cyfluthrin.

Plan of work FY96: Cooperate with industry to conduct further testing of chemicals that are being developed for the post-harvest market.

8. Specific Objective: Determine residual efficacy of cyfluthrin and deltamethrin as grain protectants.

Progress FY95: Both materials have been evaluated and results have been published. Either protectant would control insect pests for a typical storage period. Combination treatments with chlorpyrifos-methyl are also effective. Data are being submitted for registration.

Plan of Work FY96: Conduct any small-scale tests necessary to complete data acquisition.

9. Specific Objective: Determine the potential for the expanded use of aeration on stored grains.

Progress FY95: Aeration slows the degradation rate of pirimiphos-methyl, lowers insect populations, and reduces the amount of insect feeding damage in stored corn. Models developed from recorded weather data indicate that aeration can be effective on corn stored in the southeastern U. S.

Plan of Work FY96: Collect data to develop models for using aeration on corn and wheat stored in various geographic regions of the U. S.

10. Specific Objective: Evaluate microbial pathogens as protectants of stored grains.

Progress FY95: Entered into cooperative agreements with ARS scientists to conduct research on microbial and fungal pathogens. Evaluated several methods of rearing Indianmeal moths on corn. None were satisfactory.

Plan of Work FY96: Continue to research rearing methods for moths so that residual testing can be conducted.

11. Specific Objective: Cooperate with industry to evaluate new aerosol fogs that are being developed for the stored product market.

Progress FY95: No new fogs are available for testing. Conducted a demonstration project in a General Mills warehouse to examine efficacy of application systems.

Plan of Work FY96: Contact industry representatives to determine if new candidate aerosols are in the developmental stage and determine the potential for registration.

12. Specific Objective: Examine sublethal affects of high temperature on insect population structure. Consider efficacy of heat as an alternative to methyl bromide fumigation.

Progress FY95: Laboratory tests indicated that there is no difference in heat susceptibility of male and female confused and red flour beetles. Insect age also did not influence the heat tolerance of adult beetles. Because there is no difference in heat tolerance due to insect sex, we expect no change in the sex ratio among the surviving beetles after a heat treatment. Therefore, normal population growth and reinfestation would be anticipated.

Plan of Work FY96: Work has been initiated to examine the effects of sublethal temperatures on the reproductive capabilities of flour beetles.

13. Specific Objective: Determine the nature and sources of insect infestations in commercial grain elevators with an emphasis on the relative importance of refugial populations in and around commercial facilities.

Progress FY95: The elevators monitored had resident insect populations inhabiting all areas of the facility. The numbers of stored-product insects collected from any one area within an elevator was typically not well correlated with insect populations in any other area of that elevator.

Plan of Work FY96: Monitor seasonal dynamics of stored-product insect activity using molecular biological techniques.

14. Specific Objective: Determine sources of insect infestation along marketing channels by using DNA fingerprinting technology.

Progress FY95: Developed a DNA fingerprinting method to identify geographical populations of stored-product insects and differentiated several populations of Indianmeal moths that were detected in stored grain. This was accomplished by finding unique DNA fingerprints for each population. Populations that originated from areas as close as 60 miles from each other could be correctly differentiated.

Plan of Work FY96: Continue work with Indianmeal moth and initiate work on lesser grain borer and flour beetles.

15. Specific Objective: Examine DNA fingerprints to detect and monitor for insecticide resistance in field populations.

Progress FY95: Screening of the Indianmeal moth for genetic fingerprints linked to resistance to the bacterial insecticide *Bacillus thuringiensis* was conducted. Characterizing the expression of messenger RNA that codes for trypsin and chymotrypsin enzymes in Indianmeal moths was also conducted.

Plan of Work FY96: Research will continue on using DNA fingerprints to detect and monitor insecticide resistance. This may eventually lead to a quick test to be used in field situations.

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TECHNOLOGY TRANSFER

IMPLEMENTING BIOLOGICAL CONTROL IN STORED GRAIN. Provided two species of hymenopteran parasitoids and mass rearing instructions to Mr. Buddy Maedgen, President, Biofac, Inc., Mathis, TX. This is one of the major companies marketing parasitoids for the control of stored-product insects. Provided information on use of parasitoids for biological control of stored grain pests to two popular magazines and also two industrial companies.

MANAGEMENT OF INSECT RESISTANCE TO BT. Continued studies to develop information needed for managing insect resistance to BT toxins. Worked with ARS, University, and Industry representatives to develop plan for coordinating and funding research. Continued ongoing studies on cross-resistance among different BT toxins, a major obstacle to some of the simpler strategies for resistance management. Conducted research on DNA fingerprinting as a method for detecting resistance in populations (success with geographic strains already has been shown).

BIOTECHNOLOGICAL APPROACHES TO CONTROLLING CORN INSECTS. Continued research discussions and exchanges of data with staff scientists from Pioneer Hi-Bred International, Inc. concerning applications of biotechnology for preservation of corn against field and storage insect pests. This work is supported in part by CRADA to ARS and research contract to Kansas State University.

RESEARCH ON SPACE TREATMENTS FOR PROCESSING FACILITIES. Conducted collaborative research with General Mills to evaluate the efficacy of pyrethrin fogs and different equipment for dispensing the fogs in processing facilities. Processors are in a transition away from use of dichlorvos as a fog treatment and methyl bromide as a periodic fumigation. Data are not available to tell us whether pyrethrin fogs are going to do the job. A trial last month in a General Mills facility in Des Moines yielded inconsistent results. This suggests that more research is needed and that this will be an important interaction with industry.

INSECT RESISTANT PACKAGING. Research on insect resistant packaging has been re-established at the U.S. Grain Marketing Research Laboratory, Grain Marketing and Production Research Center, following closure of the Savannah Laboratory. Collaborative work is in progress on raisins, pet foods, and baby cereals with Ralston Purina, Quaker Oats Co., H. J. Heinz, Cambridge Chemical Company, Container Corporation of America, and the Armed Forces Pest Management Board. A CRADA with Ralston Purina has been extended and funds were added.

PACKAGES WERE TESTED FOR INSECT RESISTANCE AND RECOMMENDATIONS WERE MADE FOR IMPROVEMENT. The results will lead to significant reductions in losses due to insect infestation in pet foods and other packages of consumer products.

EXPERT SYSTEM FOR STORED GRAIN MANAGEMENT. Developed and released "Stored Grain Advisor" an expert system for stored grain management. This expert system is being used in 100 facilities storing over 100 million bushels of grain in Oklahoma. Montana State University has trained over 240 grain managers using this system. Stored Grain Advisor will also be used by the Kansas State University Extension Service to train county agents and farmers.

INSECT MONITORING TECHNOLOGIES. Completed three years of pilot scale testing of an automated system for monitoring insects infesting 10 bins of wheat stored on five Kansas farms. Acoustical sensors on cables were shown to detect insect infestations earlier and at lower densities than conventional grain sampling. Initiated collaboration and worked with The Rolfes Company to develop a cable with acoustical sensors suitable for commercial production and marketing.

TRAINING CONFERENCE FOR EXTENSION SERVICE. Hosted IV National IPM Training Conference on Pest Management in Stored Grain for state extension service and grain industry personnel September 18-21, 1994.

HANDBOOK DESCRIBING PESTS OF STORED CORN SOON TO BE PUBLISHED. Unit scientists authored chapters in a handbook of corn insects. The handbook will assist growers, elevator operators, and grain handlers in pest insect identification.

INVENTION DISCLOSURES

An Invention Disclosure (D.N. 0206.94) entitled "Malathion resistant strain of a predaceous bug (Heteroptera: Anthocoridae) has been filed with the Office of Technology Transfer, September 12, 1994. Current status: Awaiting patent application preparation.

An Invention Disclosure entitled "Antibody to maize weevil egg plug proteins" has been filed with the Office of Technology Transfer, June 7, 1995.

Invention disclosure entitled "Recombinant chitinase and use thereof as a biocide" has been filed. Kramer, K. J., S. Muthukrishnan, H. K. Choi, L. Corpuz, and B. Gopalakrishnan. U. S. Patent Application #08 / 224,987.

CUSTOMER SERVICE ACTIVITY

Met with representatives of B.t. industries to discuss research needs and strategies for managing insect resistance to *Bacillus thuringiensis* December 14, 1994.

Met with Dept. of Defense personnel to review pest management research findings applicable to DoD pest problems January 30-February 1, 1995. Provided information on the revision of Technical Information Memorandum No. 27, *Stored-Product Pest Monitoring Methods*, for the Armed Forces Pest Management Board.

Met with representatives of CSRS and scientists at Oklahoma State University to discuss transfer of technologies to Oklahoma Extension and industry May 8, 1995.

Provided advice to the International Atomic Energy Agency, the UN, the Florida Dept. of Agriculture, and Atomic Energy, Ltd. of Canada regarding irradiation for stored grain insect control.

Discussions were held with Kansas State University extension personnel regarding on-farm research results that they will incorporate into extension activities.

Discussions were held with W. Pursley, American Institute of Baking, R. Richardson, General Mills, and G. Swoyer, Industrial Fumigant Co. regarding alternatives to methyl bromide and dichlorvos.

Discussions and planning for development of an improved pheromone trap and lures for stored-product Coleoptera were held with David Mueller of Insects Limited and Bill Lingren of Tre'ce', Inc. Consultations were held with Joan Fisher of IPM Technologies, Inc. on possible cooperative research on the development of insect pheromone lures.

